

CLAIMS

1. A method for preparing a cytotoxic lymphocyte characterized in that the method comprises the step of carrying out at least one step selected from
5 induction, maintenance and expansion of a cytotoxic lymphocyte using a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of fibronectin, a fragment thereof or a mixture thereof.
- 10 2. The method according to claim 1, wherein the cytotoxic lymphocyte highly expresses an interleukin-2 receptor as compared to a cytotoxic lymphocyte prepared in the absence of fibronectin, a fragment thereof or a mixture thereof.
- 15 3. The method according to claim 1, wherein the cytotoxic lymphocyte contains CD8-positive cell in a higher ratio as compared to a cytotoxic lymphocyte prepared in the absence of fibronectin, a fragment thereof or a mixture thereof.
- 20 4. The method according to claim 1, wherein an expansion fold is higher as compared to that of a method for preparing a cytotoxic lymphocyte in the absence of fibronectin, a fragment thereof or a mixture thereof.
- 25 5. The method according to any one of claims 1 to 4, wherein a cytotoxic activity is enhanced or high cytotoxic activity is maintained as compared to a

cytotoxic activity of a cytotoxic lymphocyte prepared in the absence of fibronectin, a fragment thereof or a mixture thereof.

- 5 6. The method according to any one of claims 1 to 5, wherein fibronectin, a fragment thereof or a mixture thereof is immobilized on a solid phase.
7. The method according to claim 6, wherein the solid phase is a cell culture equipment or a cell culture carrier.
- 10 8. The method according to claim 7, wherein the cell culture equipment is a petri dish, a flask or a bag, and the cell culture carrier is beads, a membrane or a slide glass.
- 15 9. The method according to any one of claims 1 to 8, wherein the cytotoxic lymphocyte is a lymphokine-activated killer cell.
- 20 10. The method according to any one of claims 1 to 9, wherein the fibronectin fragment is a polypeptide (m) comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 8 of Sequence Listing, or a polypeptide (n) comprising at least one amino acid sequence having substitution, deletion, insertion or addition of one or the plural number of amino acids in any one of said amino acid sequences, wherein the polypeptide (n) has a function equivalent to that of said polypeptide (m).
- 25 11. The method according to claim 10, wherein the fibronectin fragment has a

cell adhesion activity and/or a heparin binding activity.

12. The method according to claim 10, wherein the fibronectin fragment is at least one polypeptide selected from the group consisting of polypeptides having any one of the amino acid sequences shown in SEQ ID NOs: 9 to 20 and 25 of Sequence Listing.

13. The method according to claim 1 which is carried out in a cell culture equipment, wherein the method satisfies the conditions of:

- (a) a ratio of the number of cells to a culture area in the cell culture equipment at initiation of culture being 1 cell/cm² to 5 × 10⁵ cells/cm²; and/or
- (b) a concentration of cells in a medium at initiation of culture being 1 cell/mL to 5 × 10⁵ cells/mL.

14. The method according to claim 13, wherein the method does not require a step of diluting a cell culture solution.

15. The method according to claim 1, wherein the method comprises carrying out at least any one of induction, maintenance and expansion of a cytotoxic lymphocyte in the presence of fibronectin, a fragment thereof or a mixture thereof in a cell culture equipment containing a medium, wherein the method comprises at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment, and wherein the culture conditions immediately after at least one step of diluting the

cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment satisfy the conditions of:

(c) a concentration of cells in the cell culture solution being 2×10^5 cells/mL to 1×10^8 cells/mL; or

5 (d) a ratio of the number of cells in the cell culture solution to a culture area in the cell culture equipment being 1×10^5 cells/cm² to 1×10^8 cells/cm².

16. The method according to claim 1, wherein the method comprises carrying out at least any one of induction, maintenance and expansion of a cytotoxic
10 lymphocyte in the presence of fibronectin, a fragment thereof or a mixture thereof in a cell culture equipment containing a medium, wherein the method comprises at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment, and wherein a total concentration of serum and plasma in the medium immediately
15 after at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment is same as that at initiation of the culture or lowered as compared to that at initiation of the culture.

20 17. A cytotoxic lymphocyte obtained by the method as defined in any one of claims 1 to 16.

18. A medicament comprising as an effective ingredient the cytotoxic lymphocyte obtained by the method as defined in any one of claims 1 to 16.

25 19. A medium for culturing a cytotoxic lymphocyte, characterized in that the

medium comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof, and that a total concentration of serum and plasma in the medium is 0% by volume or more and less than 5% by volume.

5 20. The method according to any one of claims 1 to 16, further comprising a step of transducing a foreign gene into a cytotoxic lymphocyte.

21. The method according to claim 20, wherein the foreign gene is transduced using retrovirus, adenovirus, adeno-associated virus or simian virus.

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22. A polypeptide having the amino acid sequence (x) shown in SEQ ID NO: 25 of Sequence Listing or an amino acid sequence (y) having deletion, insertion, addition or substitution of one or the plural number of amino acids in the amino acid sequence (x), wherein the polypeptide having the amino acid sequence (y) has a function equivalent to that of the amino acid sequence (x).

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23. A nucleic acid encoding the polypeptide of claim 22.

24. The nucleic acid according to claim 23, wherein the nucleic acid comprises (1) a DNA comprising the nucleotide sequence shown in SEQ ID NO: 26; (2) a DNA comprising a nucleotide sequence having deletion, substitution, insertion or addition of one or the plural number of nucleotides in the nucleotide sequence shown in SEQ ID NO: 26, wherein the DNA encodes a polypeptide having a function equivalent to that of the polypeptide encoded by the DNA (1); or (3) a DNA which hybridizes to a DNA comprising the

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nucleotide sequence shown in SEQ ID NO: 26 under stringent conditions, wherein the DNA encodes a polypeptide having a function equivalent to that of the polypeptide encoded by the DNA (1).